Biochemical Pharmacology, Vol. 32, No. 23, pp. 3595–3600, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

IN VIVO HYDROXYLATION OF ^{3}H-ACETANILIDE-EVALUATION OF A NEW RADIOSPIROMETRIC METHOD IN THE RAT

MOHAMED TOUTOUNGI, HANS-UELI BIERI, PHILIPP HUGUENIN†, GEORG KARLAGANIS, TSCHING TSE ZENG‡ and JOHANNES BIRCHER*

Department of Clinical Pharmacology, University of Berne, Switzerland

(*Received* 9 *May* 1983; *accepted* 1 *July* 1983)

Abstract—The proposed *in vivo* methodology for the investigation of hydroxylation rates consists of of the i.v. administration of tritrated substrates and the collection of tritiated water (HTO) from exhaled air as a measure of HTO accumulation in body water. Specifically, HTO was assessed in exhaled water after i.v. administration of ^{3}H-acetanilide. Over a wide range the half lives of accumulation of HTO in exhaled water (T_{50}) were almost identical with the half lives of elimination of ^{3}H-acetanilide in blood, evaluated by an inverse isotope dilution method ($r = 0.96$, N = 18). Average T_{50} amounted to 29 min in controls, was reduced to 20 min after enzyme induction by phenobarbital or 3-methylcholanthrene, and prolonged to 45, 46 and 66 min after bile duct ligation, portacaval shunt and a single dose of ethanol, respectively. It is concluded that the chosen pharmacokinetic approach corrects for the NIH-shift and the results adequately reflect changes in acetanilide hydroxylation related to enzyme induction or inhibition and to liver pathology.

It is well known that rates of drug metabolism may be subject to many genetic, environmental or pathological influences. The clinical relevance of these changes in individual subjects or experimental animals, however, is as yet insufficiently documented because only few *in vivo* methods for the measurement of rates of drug metabolism are available. They are generally based upon the measurement of plasma or blood clearances, and upon the collection of urinary metabolites [1]. Alternatively, after administration of an appropriately labeled test compound, exhaled $^{14}CO_2$ has been assessed [2]. Only the latter procedure, and the collection of urinary metabolites are noninvasive. In view of these limitations of the presently available procedures, the search for other radiospirometric methods including the measurement of other metabolic routes appears warranted.

Hydroxylation of a drug which is labeled with ^{3}H at the specific site of its metabolism may result in the formation of tritiated water (HTO) [3]. *In vivo*, HTO is expected to distribute itself within the body water [4]. "Distilled" body water may be sampled in the exhaled air. Theoretically, the possibility therefore arises that measurements of the specific activity of HTO in exhaled water could be used to assess rates of drug metabolism. Care, however, has to be taken in regard to isotope effects [5, 6] and the NIH-shift [7]. If these difficulties could be overcome the rate of HTO-accumulation might be used as an indicator for the hydroxylation rate.

This paper describes in the rat the methodology of HTO-collection in breath and applies it to an assessment of acetanilide hydroxylation in different conditions. This drug was chosen as test compound because several important aspects of its metabolism have already been investigated [5–11]. The problems resulting from the NIH-shift and from possible isotope effects could, therefore, be compared with published data.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (Süddeutsche Versuchstierfarm, Tuttlingen, F.R.G.) weighing 190–300 g were used. They were housed in groups of 4–6 animals at constant temperature (24°) and humidity (47%) and with an artificial 12-hr day–night rhythm. Tap water and food pellets (1324 Altromin, Altromin International, Lage F.R.G.) were allowed *ad libitum* up to the time of the experiments.

Chemicals. Tritiated water (HTO, the Radiochemical Centre, Amersham, U.K.) with a specific activity of 5 mCi/ml (185 MBq/ml) was diluted with physiological saline to 100 μCi/ml and this stock solution was stored at 4°. Generally labelled acetanilide was prepared from (ring-G-^{3}H)aniline (the Radiochemical Centre, Amersham) by reaction with acetic anhydride in pyridine and was shown to be radiochemically pure by TLC (silica gel; benzene/methanol, 95 : 5 (v/v). The specific activity was 133 mCi/mol. This means, that among 10^6 molecules of acetanilide there are only an average of 6.4 tritium atoms. Stochastically, therefore, the number of doubly labelled acetanilide molecules can be neglected and for practical purposes it was assumed that the

* Address for correspondence: Department of Clinical Pharmacology, Murtenstrasse 35, CH-3010 Berne, Switzerland.

† Present address: National Cancer Institute NIH, Building 37, Room 6D24, Bethesda, MD 20205, U.S.A.

‡ Recipient of a Swiss National Exchange Grant, present address: Pharmacy School of Sichuan, Medical College, Chengdu, Sichuan, China.

Supported by the Swiss National Science Foundation.

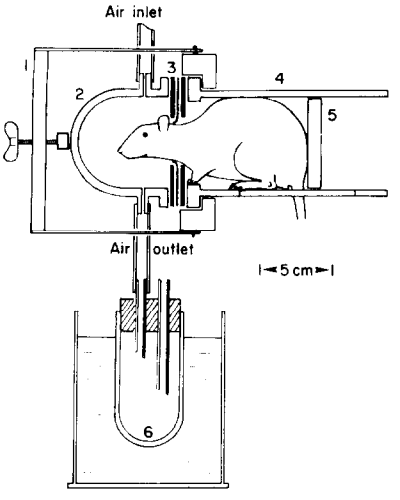

Fig. 1. Restraining cage with head chamber for collection of exhaled water vapor in the rat. The head of the rat is isolated in the head chamber by a diaphragm. Dry air is introduced into the head chamber by the air inlet, and exhaled water vapor is led through a cold trap of about −70°. The numbers refer to the following times: (1) Stirrup with screw to fasten head chamber (2) and rubber collars (3) against restraining cage (4). (2) Semispherical glass 7 cm dia, 190 ml vol) with in- and outlet forming the head chamber. (3) Set of rubber dams (collars), consisting of two 3 mm rubber disks with central openings (diameter of openings 26, 28, 30 or 32 mm, as required). Sandwiched between these disks a thin rubber membrane derived from a surgical glove with central opening (diameter of opening 24, 26, 28 or 30 mm, as required) gently fitting around the neck of the rat. (4) Cylindrical restraining cage (plexi-glass) with slots for legs and tail of the rat. (5) Round caudal end plate with lateral screws, adjustable to the size of the rat. (6) Cold trap formed by a glass vial (diameter 3.5 cm, length 9.5 cm) and a perforated rubber stopper with Teflon.

labelled acetanilide molecules carried 1 tritium atom randomly distributed on the benzene ring. Unlabelled acetanilide (purum) and 3-methylcholanthrene (puriss) were purchased from Fluka AG, Buchs, SG, Switzerland. The scintillator, Lumagel, was obtained from Lumac System AG, Basle, Switzerland and Sicapent (P_2O_5) from Merck Darmstadt, F.R.G. Phenobarbital, arachidic oil and ethanol 100% were U.S.P. grade.

Investigated animal models. The study of HTO metabolism was carried out in rats without pretreatment. For enzyme induction, phenobarbital (in 3–4 ml of physiological saline) was administered intraperitoneally at doses of 80 mg/kg for 3 consecutive days. The controls received saline only. Acetanilide metabolism was investigated on the 4th day. Enzyme induction with 3-methylcholanthrene was carried out for 4 consecutive days at a daily intraperitoneal dose of 20 mg/kg in 1–1.5 ml of arachidic oil. The controls received arachidic oil only. Acetanilide metabolism was investigated on the 5th day. End-to-side portacaval shunts were made as described previously [12] and the rats were studied 3–4 weeks later. The controls were subjected to an appropriate sham operation. Bile duct ligation in ether anesthesia was carried out by placing two ligatures at the common duct and cutting it between them. The sham operated controls underwent a similar amount of manipulation of the common duct but without ligature or incision. The studies of acetanilide metabolism were carried out 48 hr after the operation. To evaluate the acute effects of alcohol, the rats received ethanol intraperitoneally at a dose of 3 g/kg diluted with 4 vol. of saline 0.9%. The controls received only saline. Acetanilide metabolism was investigated 30 min after the injection of alcohol. Since the results of the different control animals were essentially the same, they were combined.

Collection of exhaled water. For the collection of exhaled water a special restraining cage had been constructed (Fig. 1). It had a head chamber, which was separated from the rest of the cage by a soft rubber diaphragm gently fitting around the neck of the animal. A stream of air was allowed to flow at a rate of 0.5 l/min through a P_2O_5 column (7 × 15 cm) in order to eliminate water. The dried air entered the inlet of the head chamber. The outlet was connected to a cold trap (−78°) consisting of a vial immersed into an isopropanol dry-ice mixture. Dimensions were chosen as to minimize dead spaces. For the collection of exhaled water the cooled vials were exchanged at intervals of 12 or 30 min depending on the specific experimental design. They were closed immediately in order to prevent contamination by condensing water and allowed to rewarm to room temperature. After careful drying of the outside with tissue paper 2 or 10 μl of the condensate were pipetted either with a 2 μl constriction pipette or with a 10 μl Eppendorf pipette into a counting vial containing 5 ml of Lumagel and were counted.

Assessment of acetanilide hydroxylation. All experiments were started at 8–9 am. The animals were weighed and placed into the breath collecting device. An intravenous infusion was started at the tail vein and a blank of expired water was obtained for 30 min. Thereafter 18 μCi corresponding to 135 nmoles of (ring-G-^{3}H)acetanilide was injected intravenously in a volume of 0.6 ml physiological saline and flushed with 2–3 ml of saline. The sample vial was exchanged every 30 min and thereby exhaled water was collected continuously for 3–4 hr. After thawing 10 μl samples of exhaled water were counted in a Packard Tri-carb 2660 liquid scintillation counter. In pilot experiments for the study of HTO recovery breath collection periods of 12 min and a 2 μl constriction pipette were used. Volumes of 10 μl of the injected solution of HTO or of acetanilide were also counted in 5 ml Lumagel. At the end of each experiment with acetanilide the rats were killed in ether anesthesia and autopsied. Shunt patency and bile duct ligation were verified and the liver removed and weighed.

Measurement of acetanilide elimination from blood. In some experiments blood was obtained from the saphenous vein at 5-min intervals for 30 min after acetanilide injection. Samples of 50–200 mg of blood were transferred by means of hematocrit capillaries into weighed plastic tubes which contained a small amount of heparin. They were weighed again, 1 ml of 5 mM potassium phosphate buffer of pH 7 was added and after mixing the samples were stored at −20°.

For the analysis the solution was thawed in a water bath at room temperature and transferred into a separatory funnel with 20 ml 5 mM potassium phosphate buffer pH 7. After addition of 60 ml of diethylether and exactly 50 mg of unlabelled acetanilide the separatory funnel was strongly shaken for about 1 min. The organic phase was washed with water and dried over anhydrous magnesium sulfate. After evaporation the residue was recrystallized once from toluene. The crystals were washed with hexane and dried under high vacuum at room temperature for 15 min. Control experiments had shown that a second recrystallization did not raise the specific activity of acetanilide. The specific activity of the recrystallized acetanilide was determined by weighing and liquid scintillation counting with Lumagel and the blood concentration of (ring-G-^{3}H)acetanilide was calculated [13].

Biochemical methods. Biochemical tests were carried out by routine clinical methods on an autoanalyser. Conjugated serum bile acids were determined by a solid phase radioimmunoassay obtained from Becton Dickinson, New York [14].

Calculations. For the calculation of acetanilide hydroxylation it was assumed that each molecule of HTO was derived from one molecule of acetanilide, i.e. that only one hydroxylation occurred per molecule of acetanilide. The tritiated water was considered to be distributed within a space approximately corresponding to the body water for which a value of 2/3 of the body wt was taken [4, 15–18]. Furthermore, the exhaled water was found to underestimate the specific activity of tritiated body water by 10.1%. Therefore the measured specific activity of exhaled tritiated water was multiplied by 66.7% of the body weight of each rat, and by 1.101 and divided by the dose. For the later, 1/5 of the injected radioactivity was taken, since it was assumed that on the average ^{3}H is equally distributed over the benzene ring and that only one hydroxylation per molecule of acetanilide occurred.

Plasma disappearance rate constants (k) for acetanilide were calculated by log–linear regression analysis. The volume of distribution (V_d) was obtained as ratio of the dose to the extrapolated plasma concentration at time zero, assuming a density of 1.0 for blood, and the clearance (CL) as a product of k and V_d. Standard statistical techniques were applied [19], and $P < 0.01$ was taken as level of significance.

RESULTS

Experiments with tritiated water (HTO). When 20 μCi of HTO were injected intravenously to the rats and exhaled water was sampled over periods of 12 min, the label rapidly appeared in exhaled water and approached a constant level after about 50 min. The time needed to reach half the maximal specific activity, obtained by linear interpolation in each experiment, was 4.7 ± S.E. 0.2 min (N = 6). The specific activity of HTO, observed for a period of 9 days fell with a half life of 76.9 ± S.E. 4.6 hr (N = 7). In experiments with HTO collection for more than 24 hr after dosing it was assumed that the distribution of the label within the organism was complete. At this time the radioactivity in exhaled

Table 1. ^{3}H-acetanilide hydroxylation measured by HTO exhalation in different experimental models ($\bar{x}$ + S.D.)

Model	N	Body weight (g)	Liver weight (g/100 g)	Maximal HTO accumulation (% of dose)	T_{50}* (min)
Controls	16	297 ± 53	4.7 ± 0.4	64.3 + 6.1	29.2 + 5.1
3-Methyl-cholanthrene	13	223 ± 38‡	5.9 ± 0.3‡	78.5 ± 6.2‡	19.9 ± 5.5‡
Phenobarbital	9	258 ± 31	5.5 ± 0.3‡	61.8 ± 8.6	20.2 ± 6.0‡
48-hr Bile duct ligation	7	248 ± 41	4.9 ± 0.4	57.2 ± 5.1	44.8 ± 12.4‡
Portacaval shunt	12	256 ± 53	2.5 ± 0.4‡	55.3 ± 9.6‡	46.3 ± 19.7†
Ethanol (3 g/kg, i.v.)	6	321 ± 35	4.4 ± 0.3	57.9 ± 7.8	65.5 ± 25.3‡

* Calculated as explained in Fig. 1. The data are not corrected for the average 4.7 min required for i.v. injected HTO to achieve 50% equilibration with exhaled water. Thus, the biologically relevant T_{50} values are shorter by an average of 4.7 min.

† Significantly different from controls by t-test: $P < 0.01$.

‡ Significantly different from controls by t-test $P < 0.001$.

Table 2. Biochemical tests in rats 48 hr after bile duct ligation and in normal controls

	48-Hr bile duct ligation*	Controls
N	7	6
SGOT (I.U.)	361 ± 118	98 ± 40
SGPT (I.U.)	316 ± 90	80 ± 64
Alkaline phosphatase (I.U.)	205 ± 49	68 ± 16
Total bilirubin (mg/dl)	3.2 ± 0.6	0.09 ± 0.03
Conjugated bile acids (μmol/l)	231 ± 133	3.7 ± 0.37

* Compared to controls all differences are significant at $P < 0.01$ (t-test).

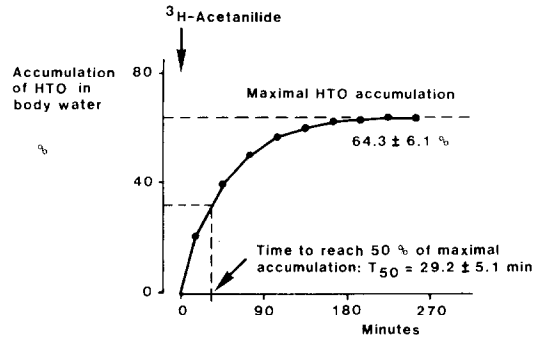

Fig. 2. Accumulation of HTO in exhaled water after intravenous administration of ^{3}H-acetanilide to the rats. The specific activity of exhaled water was corrected for body weight, vapor pressure and dose as detailed in the method section. Thus the ordinate represents the percentage of the dose which could be recovered in the body water in the form of HTO. The time to reach 50% of the maximal accumulation, designated as T_{50}, was obtained in each case by linear interpolation. The line represents mean values, the observations are given as $\bar{x} \pm$ S.D. (N = 16).

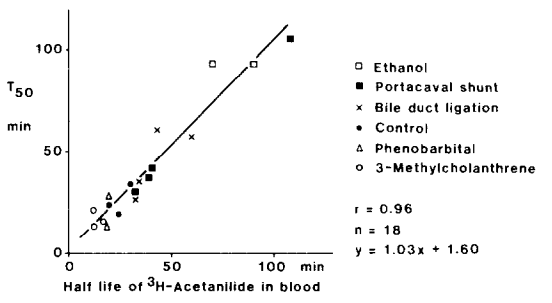

Fig. 3. Correlation between half lives of acetanilide in blood and time to reach 50% of maximal accumulation of HTO in exhaled water (T_{50}). The slope of the regression line which is close to unity indicates that both procedures measure essentially the same process. If each value for T_{50} is corrected by substracting the time for intravenously injected HTO to reach half the equilibrium with exhaled water (averaging 4.7 min), the slope remains unchanged and the intercept changes to −3.1 min, which is not significantly different from zero.

water was 10.1 ± S.E. 0.8% lower than the specific activity of HTO in plasma water (26 samples in 7 rats).

Experiments with acetanilide in the rat. When ^{3}H-labelled acetanilide was administered to the rats, accumulation of HTO in exhaled water occurred nonlinearly (Fig. 2) and approached a maximum after 2–5 hr suggesting that at this time hydroxylation of acetanilide had ceased, presumably because it was completely metabolized. The period of time required to reach 50% of the maximal accumulation of HTO in exhaled water was thought to represent the sum of time for 50% of the hydroxylation to take place and for the label to distribute itself into the exhaled water. Therefore, as a first approach this period of time, designated as T_{50}, was taken as an inverse measure of the hydroxylation rate. As shown in Table 1, maximal HTO accumulation in control rats averaged 64.3% and T_{50} 29.2 min.

The different experimental groups (as detailed in Table 1) show the expected increases in liver weight after enzyme induction and the decrease after portacaval shunt [12]. Biochemical tests obtained after bile duct ligation (Table 2) revealed that the liver exhibited signs of cholestasis typical for this operation [20]. Maximal HTO accumulation was significantly increased after 3-methylcholanthrene treatment and decreased after portacaval shunt. In the other models it remained unchanged. Average T_{50} was reduced to 19.9 and 20.2 min after enzyme induction with 3-methylcholanthrene and phenobarbital, respectively. It was prolonged to 44.8 min after bile duct ligation, to 46.3 min after a portacaval shunt and to 65.5 min after pretreatment with a dose of ethanol.

In order to verify the validity of the measurements in exhaled water, blood concentrations of acetanilide were also assessed. The results are summarized in Table 3. As expected, total blood clearances were increased after enzyme induction and reduced in the three models designed to show reduced rates of acetanilide hydroxylation. The relationship between acetanilide half lives and T_{50}, simultaneously obtained in the same animals is shown in Fig. 3. The correlation is linear over a wide range and the slope of the regression line, which approaches unity and nearly goes through the origin, suggests that T_{50} represents a valid estimate of acetanilide hydroxylation. T_{50} was also evaluated against the total blood clearance (CL). The reciprocal of T_{50} was highly

Table 3. Pharmacokinetics of ^{3}H-acetanilide in the rat ($\bar{x}$ − S.D.)*

Treatment groups	N	Body weight (g)	Liver weight (g/100 g)	k_{el}† (min^{-1})	Vd† (ml/100 g)	CL_{tot}† (ml/min/100 g)
Controls	3	335 ± 58	4.7 ± 0.7	0.028 ± 0.006	48 ± 31	4.2 ± 1.3
3-Methylcholanthrene	3	243 ± 11	5.6 ± 0.2	0.051 ± 0.010	143 ± 33	8.0 ± 1.0
Phenobarbital	2	268	5.5	0.037	172	6.5
Portacaval shunt	4	280 ± 84	2.6 ± 0.6	0.014 ± 0.005	121 ± 41	2.0 ± 1.1
48-hr Bile duct ligation	4	263 ± 52	5.1 ± 0.3	0.017 ± 0.004	153 ± 26	2.6 ± 0.6
Ethanol (3 g/kg)	2	308	4.4	0.009	84	0.7

* All experiments had simultaneous measurements of HTO exhaltation and are also included in Table 1.
† For pharmacokinetic calculations see Materials and Methods.

correlated with the clearance. (T_{50}^{-1} = 0.0071 CL ± 0.0077, r = 0.92, N = 18; in this equation T_{50} is expressed in min and CL in ml/min/100 g).

DISCUSSION

The data obtained in this study show that the specific activity of HTO in exhaled water may be used to assess the rate of ^{3}H-acetanilide hydroxylation *in vivo*. The results of this new radiospirometric technique apparently are consistent with conventional methods to assess acetanilide disposition. Furthermore, when tested in different experimental models the findings were similar to those with the aminopyrine breath test [21]. Nevertheless, the obstacles to proper interpretation of the data cannot be overlooked. The physicochemical and physiological limitations of the procedure have to be considered in detail.

Collection of exhaled HTO. Sampling of exhaled water without contaminations was a problem. By trial and error the following conditions were found to be essential: excreta should not come into contact with the sampled atmosphere, since they may contain large amounts of labeled metabolites and bacteria, which may transform them into volatile compounds. Contamination of the exhaled water could be avoided best by a separation of the atmosphere at the level of the animals neck. Deadspaces had to be minimized in order to achieve a rapid response of the system to metabolic processes, allowing for short equilibrium times. The apparatus as shown in Fig. 1 fulfills the above criteria to a large measure, but at the expense of an immobilization of the animal.

Furthermore, assessment of radioactivity in exhaled water to measure specific activity of HTO in body water also is possible only with certain limitations. The small samples of exhaled water demand attention to details in regard to handling of the cold trap to avoid contamination by condensed water from the outside, and to the pipetting of very small volumes (2–10 μl). A certain delay from the moment when HTO has been injected intravenously to its equilibration with the expired water is unavoidable. Some delay may also result from the equilibration of the water vapor within the head chamber and the tubing. After intravenous injection of HTO it took an average of 4.7 min to reach 50% of the maximal level of specific activity in exhaled water. Consequently, biological half lives will be overestimated by this amount and the time course of *in vivo* processes very rapidly leading to HTO formation cannot be measured accurately with this approach.

Once distributed within the organism, HTO will be eliminated from the body as part of the normal turnover of body water. In rats, water intake, metabolic water production, growth, evaporation and excretion need to be considered. Since these factors vary with the age and living conditions of the experimental animals, the calculated half life of 76.9 hr is applicable only to the investigated rats and may not be valid under other circumstances [4]. Nevertheless, the order of magnitude suggests that a correction for losses need be applied only during experiments exceeding several hours. Exhalation of water during the experiments is not considered to be a significant source of HTO loss, because total water recovery in the apparatus did not exceed 150 μl within 4 hr, which is less than 1‰ of the body water.

The lack of agreement between the specific activity of HTO in exhaled water and plasma had already been noted [22]. A convenient explanation for this observation is the lower vapor pressure of HTO compared to H_2O at 37° [22]. If HTO in exhaled water is used to assess HTO in body water it appears appropriate, therefore, to multiply the results with a correction factor of 1.101.

Assessment of acetanilide hydroxylation. Since the investigated chemical reaction involves the abstraction of a ^{3}H-atom, isotope effects might be expected. In the case of acetanilide, however, hydroxylation is thought to be initiated by the formation of an arene oxide [6], which rearranges thereafter to yield 4-hydroxyacetanilide and HTO. In this sequence of events the rate limiting step is assumed to reside in the initial formation of the arene oxide [6]. This represents the introduction of an oxygen atom into the benzene ring, which as a result of the neighboring ^{3}H-atom may be subject only to a secondary isotope effect. The latter is assumed to be negligible. The molecular rearrangement of the arene oxide eventually leading to 4-hydroxyacetanilide and to HTO is likely to exhibit isotope effects, but it will not be rate limiting and therefore can also be neglected. This view is consistent with published observations [5, 6] suggesting that the isotope effect of acetanilide hydroxylation is minimal. On this basis we assumed that, as a first approximation the rates of acetanilide hydroxylation, observed in this study, are representative for labelled as well as for unlabelled test compound.

Another complication of the chosen experimental design is related to the NIH-shift, i.e. the observation, that the molecular rearrangement of the arene oxide may not lead to stoichiometrically corresponding amounts of 4-hydroxyacetanilide and HTO. It has been reported that in the normal rat about 40% of the label may be retained on the benzene ring, presumably in the *m*-position [10]. This figure nicely agrees with the average maximal accumulation of HTO of 64.3% found in the control group. The previously reported decrease in NIH-shift after pretreatment with 3-methylcholanthrene [10] is reflected in our experiments by the increase in maximal HTO accumulation to 78.5%. This decrease in NIH-shift, however, suggests that hydroxylation of acetanilide by cytochrome P-448 may occur through a different mechanism. Whether or not an isotope effect should be expected under these circumstances is unknown [6]. The biochemical basis for an increase in NIH-shift in rats with a portacaval anastomosis is as yet unknown.

In view of this variability of the NIH-shift, a method of data analysis had to be found which circumvented the problems of incomplete and unpredictable recovery of HTO in body water and exhaled air. The calculation of a half time for the appearance of the label, the T_{50} seemed to be a simple and appropriate solution, because in principle its validity is not related to the extent of the NIH-shift. The T_{50} is thought to represent the time when 50% of the acetanilide subject to the hydroxylation pathway had

been hydroxylated, and therefore when 50% of the HTO (eventually formed as a result of hydroxylation) had appeared in the body water. The measured value in addition includes the time needed for the newly formed HTO to distribute itself into exhaled water. The half time for this latter process was found to average 4.7 min. Despite this error the close agreement between the T_{50} and the acetanilide half lives in blood is strong evidence for the contention that the T_{50} values may for practical purposes be regarded as half lives of acetanilide hydroxylation.

The chosen test compound, (ring-G-^{3}H) acetanilide might also form HTO if hydroxylation had occurred at any other position of the benzene ring. Since *p*-hydroxylation is the major metabolic pathway [8, 11] other hydroxylations have been neglected. Nevertheless, if this method is to be applied on a wider scale, the use of [4-^{3}H]acetanilide would be preferable.

The importance of the findings of this study are not limited to acetanilide hydroxylation, but may be useful for the investigation of other hydroxylation reactions, particular those which have an arene oxide as an intermediary stage and therefore are unlikely to be subject to relevant isotope effects. One potential major advantage of the method consists in the fact, that no blood sampling is required. Consequently, apart from the restraint and the inhalation of dry air, the physiology of the animals is not disturbed. Repeated studies of the hydroxylation rates are therefore possible, e.g. during toxicity testing. If applied to man the non-invasive nature of the method and its application to a wide range of different hydroxylation reactions render it interesting for clinical research. The long biological half life of the label is related to the HTO itself and in man is approximately 5–11 days [19]. If 30 μCi of the test compound are applied, the activity in exhaled water is sufficient for counting and the absorbed dose does not exceed 1 to 2 mrad.

Acknowledgements—The authors sincerely acknowledge the valuable counsel of Prof. H. Bachofen and the technical assistance of Mr. O. Aeby, Miss T. Gehrig, Mr. K. T. Krusch, Mr. E. Roth and Miss A. Wyler. The secretarial help of Miss H. Rauch is appreciated.

REFERENCES

1. J. Bircher and R. Preisig, in *Concepts in Drug Metabolism. Part B* (Eds. B. Testa and P. Jenner), p. 377. Marcel Dekker, New York (1981).
2. J. Bircher, in *Principles of Radiopharmacology* (Eds. L. G. Colombetti), p. 179. CRC Press, Boca Raton, FL (1979).
3. M. Wenzel, H. Kleucker and P. E. Schulze, *Z. Naturforschg.* **21b**, 1178 (1966).
4. C. R. Richmond, W. H. Langham and T. T. Trujillo, *J. Cell. comp. Physiol.* **59**, 45 (1962).
5. M. Tanabe, D. Yasuda, J. Tagg and C. Mitoma, *Biochem. Pharmac.* **16**, 2230 (1967).
6. J. E. Tomaszewski, D. M. Jerina and J. W. Daly, *Biochemistry* **14**, 2024 (1975).
7. G. Guroff, J. W. Daly, D. M. Jerina, J. Renson, B. Witkop and S. Udenfriend, *Science, N.Y.* **157**, 1524 (1967).
8. B. B. Brodie and J. Axelrod, *J. Pharmac. exp. ther.* **94**, 29 (1948).
9. S. Undenfriend, P. Zaltzman-Nirenberg, J. Daly, G. Guroff, C Chidsey and B. Witkop, *Archs. Biochem, Biophys.* **120**, 413 (1967).
10. J. Daly, D. Jerina, J. Farnsworth and G. Guroff, *Archs. Biochem. Biophys.* **131**, 238 (1969).
11. J. D. Baty and P. R. Robinson, *Clin. Pharm. Ther.* **21**, 177 (1977).
12. R. Herz, V. Sautter, F. Robert and J. Bircher, *Eur. J. clin. Invest.* **2**, 390 (1972).
13. J. Sacks, *Physical Techniques in Biological Research*, Vol. II, p. 44. Academic Press, New York (1956).
14. E. Minder, G. Karlaganis, U. Schmid, P. Vitins and G. Paumgartner, *Clin. Chim. Acta* **92**, 177 (1979).
15. F. A. O. Udekuru, D. D. Kozoll and K. A. Meyer, *J. Nucl. Med.* **4**, 60 (1963).
16. E. A. Pinson, and W. H. Langham, *J. appl. Physiol* **10**, 108 (1957).
17. C. R. Richmond, W. H. Langham and T. T. Trujillo, *J. Cell. comp. Physiol.* **59**, 45 (1962).
18. J. M. Foy and H. Schnieden, *J. Physiol.* **154**, 169 (1960).
19. L. Sachs, *Angewandte Statistik*, 4th edn. Springer Berlin (1974).
20. F. Schaffner, P. G. Bacchin, F. Hutterer, H. H. Scharnbeck, L. L. Sarkozi, H. Denk and H. Popper, *Gastroenterology* **60**, 888 (1971).
21. B. H. Lauterburg and J. Bircher, *J. Pharmac. exp. ther* **196**, 501 (1976).
22. M. M. Popov and F. I. Tazetdinov, *Kernenergie, DDR Berlin* **3/61**, 201 (1961).